# Hematologic and hemorheological determinants of resting and exercise-induced hemoglobin oxygen desaturation in children with sickle cell disease

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## ABSTRACT

The aim of the study was to determine the factors associated with resting and exercise-induced hemoglobin oxygen desaturation. The well-established six-minute walk test was conducted in 107 sickle cell children (50 with sickle hemoglobin C disease and 57 with sickle cell anemia) at steady state. Hemoglobin oxygen saturation was measured before and immediately after the six-minute walk test. Blood samples were obtained on the same day to measure hematologic and hemorheological parameters. Exercise-induced hemoglobin oxygen desaturation was defined as a drop in hemoglobin oxygen saturation of 3% or more at the end of the six-minute walk test compared to resting levels. No children with sickle hemoglobin C disease, but approximately 50% of children with sickle cell anemia showed mild or moderate oxygen desaturation at rest, which was independently associated with the percentage of reticulocytes. Exercise-induced hemoglobin oxygen desaturation was observed in 18% of children with sickle hemoglobin C disease and 34% of children with sickle cell anemia, and was independently associated with the six-minute walk test, acute chest syndrome rate and the strength of red blood cell aggregates in children with sickle cell anemia. No association was found in children with sickle hemoglobin C disease between exercise-induced hemoglobin oxygen desaturation and the measured parameters. Hemoglobin oxygen desaturation at rest was common in children with sickle cell anemia but not in children with sickle hemoglobin C disease, and was mainly associated with greater hemolysis. Physiological strain during exercise and red blood cell aggregation properties may predict the occurrence of exercise-induced hemoglobin oxygen desaturation in children with sickle cell anemia.

## Introduction

Hemoglobin oxygen desaturation at rest[1-7] and exercise-induced hemoglobin oxygen desaturation (EIHOD)[6,7] are common in sickle cell disease (SCD). Cellular activation and abnormal vascular cell adhesion in SCD are caused by resting hemoglobin oxygen desaturation[8] which is associated with an increased risk for vaso-occlusive crises,[9] stroke[10] and elevated tricuspid regurgitation velocity,[6,7] suggesting a role for hemoglobin oxygen desaturation in the occurrence of these complications.[11] This is supported by the fact that hemoglobin oxygen desaturation induced by a six-minute walk is associated with higher tricuspid regurgitation velocities in SCD children.[6]

Hemoglobin oxygen desaturation may be related to the rightward shift of the oxyhemoglobin dissociation curve due to the decreased affinity of sickle hemoglobin[12] which is caused by an increased content of erythrocyte 2,3-bisphosphoglycerate.[13] There is also growing evidence to suggest that hemoglobin oxygen desaturation at rest and EIHOD are independently associated with anemia[1,5,11,14] and hemolysis.[1,5,6,11,14,15] It has been proposed that chronic hemolysis could promote pulmonary vasculopathy that could cause ventilation-perfusion mismatching and limit oxygen uptake by hemoglobin.[16] Intrinsic lung disease has been suggested to participate in hemoglobin oxygen desaturation at rest or EIHOD, but several studies have failed to detect such an association.[2,6,11]

Sickle cell disease is characterized by severe hemorheological abnormalities that play a role in the pathophysiology of several acute and chronic complications.[16-24] Experimental work in non-SCD subjects and mathematical modeling strongly suggest that hemorheological impairment may contribute to hemoglobin oxygen desaturation at rest.[25] Hematocrit and red blood cell (RBC) deformability have been demonstrated to modulate pulmonary diffusing capacity.[25,26] In endurance-trained athletes without SCD, blood rheology is

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suspected to participate in EIHOD, with hypoxemic athletes having more rigid RBC than those without a reduction in hemoglobin oxygen saturation during exercise.[27,28] A similar observation has been documented in exercising horses, with hypoxemic horses exhibiting decreased RBC deformability and increased RBC aggregation.[27,29] These RBC rheological abnormalities may affect the recruitment of pulmonary capillaries, hence increasing ventilation-perfusion mismatching and leading to a reduction in oxygen saturation.[27,30] However, the association between hemorheological abnormalities and resting or exercise-induced hemoglobin oxygen desaturation has never been studied in the context of SCD.

The aim of the present study was to evaluate such associations in sickle-hemoglobin C disease (SC) and homozygous sickle cell anemia (SS) children at steady state. Our study confirms that hemoglobin oxygen desaturation at rest is common in SS children, but not in SC children, and is mainly associated with higher hemolytic rate. EIHOD did occur in SC, but is more frequent in SS children. The physiological strain during exercise and RBC aggregation properties may be involved in the occurrence of EIHOD in SS children.

## Design and Methods

### Patients

The study included 107 SCD children (SC n=50, SS n=57; 8-16 years old) at steady state, i.e. no blood transfusions in the previous three months, absence of acute episodes (infection, vaso-occlusive crises (VOC), acute chest syndrome (ACS), stroke, priapism, splenic sequestration) for at least one month before inclusion in the study.

Charts were retrospectively reviewed by 3 physicians to recognize all ACS and VOC episodes from birth to the time of blood sampling based on previously described criteria.[22] The rates of ACS and VOC were calculated for each child by dividing the total number of ACS or painful VOC episodes by the number of patient-years.[22,31]

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Regional Ethics Committee (CPP Sud/Ouest Outre Mer III, Bordeaux, France; registration number 2009-A00211-56). Children and their parents were informed of the purpose and procedures of the study, and gave written consent. Details for pulmonary function tests and asthma screening are available in the *Online Supplementary Appendix*.

### Hematologic and hemorheological measurements

Blood was drawn by venipuncture into EDTA tubes and used to measure hematologic parameters.[22] Serum lactate dehydrogenase and total bilirubin concentrations were determined by standard biochemical methods.

Hemorheological parameters were measured immediately after sampling and after full re-oxygenation of the blood.[32] Blood viscosity was measured at native hematocrit (Brookfield DVII+ cone-plate viscometer, CPE40-spindle, ≈25°C, 90 $s^{-1}$). Red blood cell deformability was determined at 37°C at two shear stresses (3 and 30 Pa) by ecktacytometry (LORCA, RR Mechatronics, Hoorn, The Netherlands). RBC aggregation was determined at 37°C via syllectometry (i.e. laser backscatter *vs.* time) (LORCA, RR Mechtronics, Hoom, The Netherlands) after adjustment of the hematocrit to 40%. The RBC disaggregation threshold, i.e. the minimal shear rate needed to prevent RBC aggregation or to breakdown existing RBC aggregates, was determined using a re-iteration procedure.[33]

### Six-minute-walk test

A self-paced six-minute walk test (6MWT) was conducted according to the guidelines of the American Thoracic Society.[34] The 6MWT is a submaximal exercise test, often used in the SCD population to determine functional status or changes in status as a result of an intervention.[35] The percentage of predicted distance was calculated according to the models of Geiger *et al.*[36] Hemoglobin oxygen saturation ($SpO_2$) was obtained by finger pulse oximetry (SureSigns VS3 No. 3000, Philips Medical System, Andover, MA, USA) before and immediately after the 6MWT. Hemoglobin oxygen desaturation at rest and after exercise was defined according to Campbell *et al.*[6] EIHOD was defined as a drop in SpO2 of 3% or more during exercise compared to the resting level.[6]

### Statistical analysis

All values were expressed as means ± SD. Univariate analyses were conducted to compare the different parameters between groups. All variables at $P<0.2$ by univariate analyses were included as covariates in the multivariate models. $P<0.05$ was considered significant. Analyses were conducted using SPSS (v. 20, IBM SPSS Statistics, Chicago, IL, USA).

Further information concerning study design and methods are available in the *Online Supplementary Appendix.*

## Results

### Comparisons between SS and SC children

Previous studies showed that hemoglobin oxygen desaturation is less frequent in sickle-hemoglobin C disease (SC) than in homozygous sickle cell anemia (SS).[1,5,11,14,37] SC and SS diseases should be considered as distinct disorders[38] and, as described elsewhere, the hemorheological and hematologic profiles in SC patients differ considerably to those in SS patients (*data not shown*).[20,22,23,39] Therefore, SC and SS children were analyzed separately. Part of the results described here were previously included in a report on the initial SAPOTILLE cohort.[22]

Compared to SC children, SS children exhibited greater VOC (SS: 0.54±1.01 *vs.* SC: 0.25±0.39; $P<0.05$) and ACS rates (SS: 0.14±0.17 *vs.* SC: 0.03±0.06; $P<0.001$). Among SS children, 21.1% were receiving hydroxyurea therapy (12 of 57 patients) compared with none in the SC group.

Sickle cell anemia children exhibited lower $SpO_2$ at rest (SS: 97.6±2.9% *vs.* SC: 99.8±0.4%; $P<0.001$) and after the 6MWT (SS: 95.1±5.2% *vs.* SC: 98.8±3.0%; $P<0.001$) and reduced total six-minute distance (SS: 459±76 *vs.* SC: 494±89; $P<0.05$) compared to SC children. No SC children, but approximately 50% of SS children had hemoglobin oxygen desaturation at rest. Immediately after the 6MWT, approximately 18% of SC children and approximately 34% of SS children exhibited oxygen desaturation (i.e. a drop in $SpO_2 \geq 3\%$). The percentage of predicted distance walked tended to be lower in SS children compared to SC children (SS: 70.0±11.4% *vs.* SC: 74.1±12.3%; $P=0.088$). None of the SCD children included in this study had to prematurely stop the 6MWT and no unexpected events occurred during the tests.

### Hemoglobin oxygen desaturation at rest is associated with hemolysis in SS children

At rest, SS children were classified into three groups as a function of $SpO_2$: no desaturation ($SpO_2 > 98\%$), mild desaturation ($95 \leq SpO_2 \leq 98\%$) and moderate desatura-

tion ($SpO_2$ < 95%).[6] Nineteen SS children had mild desaturation, and 8 had moderate desaturation at rest (Table 1). There was no difference in distribution for gender, α-thalassemia, age, distance walked, and percentage of predicted distance between the three SS subgroups. There was no difference in frequency of patients on hydroxyurea therapy between the three subgroups, but *P* was less than 0.20 (ANOVA).

Comparison of the hematologic parameters between the three SS subgroups showed that RBC counts ($P<0.05$) were lower in the moderate desaturation group compared to the no desaturation subgroup. Hemoglobin and hematocrit levels were lower in the mild ($P<0.05$) and moderate desaturation groups ($P<0.05$ and $P<0.001$, respectively) compared to patients with no desaturation. Reticulocyte counts ($P<0.001$), lactate dehydrogenase ($P<0.05$) and total bilirubin ($P<0.05$) were higher in the moderate desaturation group compared to the two other groups. No difference was found between the three groups for fetal hemoglobin, leukocytes, platelets, mean cell volume, or mean cell hemoglobin levels.

There was no difference in hemorheological parameters between the three groups, except for RBC deformability, which was lower in the mild ($P<0.01$) and moderate ($P<0.001$) desaturation groups compared to patients with no desaturation. RBC deformability was also lower in the moderate desaturation group compared to the mild desaturation subgroup ($P<0.05$ and $P=0.054$ at 3 and 30 Pa, respectively). There was no difference in RBC disaggregation threshold, RBC aggregation index or VOC rates between the three groups, but *P* was less than 0.20 by ANOVA. There was no difference in ACS rates between the three groups.

An ordinal multivariate logistical model was used to test the parameters independently associated with resting hemoglobin oxygen desaturation in SS children, and included hydroxyurea treatment as factor and hemoglobin, percentage of reticulocytes, RBC deformability at 30 Pa, RBC aggregation index, RBC disaggregation threshold, and VOC rate as covariates. We used the percentage of reticulocytes in the model instead of lactate dehydrogenase or total bilirubin as several studies strongly suggest that percentage of reticulocytes reflects hemolysis more accurately than do the two other markers measured in our study.[40,41] RBC count, hematocrit, lactate dehydrogenase, total bilirubin and RBC deformability at 3 Pa were not included in the model to avoid co-linearity effects with hemoglobin level, percentage of reticulocytes or RBC deformability at 30 Pa.

The overall model was highly significant ($\chi^2=36.92$; degrees of freedom (df)=7; $P<0.001$). However, only the percentage of reticulocytes was significantly associated with the resting hemoglobin oxygenation desaturation classes (OR: 1.19; 95%CI: 1.03-1.38; $P<0.05$). To further assess a possible role of anemia, a second ordinal multivariate logistical model was used with the same previous covariates, but excluding the percentage of reticulocytes. The model was still highly significant ($\chi^2=31.46$; df=6; $P<0.001$), but none of the included parameters was significant (*P* for hemoglobin was 0.51). Because hemoglobin and percentage of reticulocytes could be related to each other, a third ordinal multivariate logistical model was used including the same covariates as in the first model, but excluding hemoglobin. The results obtained with this model were comparable to those obtained with the first (*data not shown*). Finally, since hydroxyurea (HU) therapy impacts sickle cell pathophysiology, the 12 SS children under HU treatment were excluded. Similar results were obtained by univariate analyses (*data not shown*). A fourth ordinal multivariate logistical model, including the same parameters as in the first model, except for HU therapy, was highly significant ($\chi^2=27.10$; df=6; $P<0.001$), and the percentage of reticulocytes remained significantly associated with the resting hemoglobin oxygenation desaturation classes (OR: 1.18; 95%CI: 1.01-1.37; $P<0.05$).

**Table 1. Comparison of hematologic, hemorheological, ACS rate, VOC rate and 6MWT parameters in SS children classified according to their level of hemoglobin oxygen saturation at rest.**

| | $SpO_2$ > 98% (n=29) | 95 ≤ $SpO_2$ ≤ 98% (n=19) | $SpO_2$ < 95% (n=8) |
|---|---|---|---|
| Sex ratio (M/F) | 13/16 | 10/9 | 5/3 |
| α-thalassemia (%) | 48 | 32 | 29 |
| Hydroxyurea (%)[§] | 31 | 11 | 13 |
| Age (years) | 11.8±2.5 | 11.4±2.2 | 10.4±2.2 |
| Walked distance (m) | 471±79 | 445±72 | 450±75 |
| Percentage of predicted distance (%) | 71.3±11.3 | 68.1±11.9 | 70.0±11.4 |
| Fetal hemoglobin (%) | 9.2±7.7 | 7.9±4.2 | 4.8±0.5 |
| Leukocytes ($x10^9$L) | 10.8±3.1 | 11.8±2.7 | 11.2±1.8 |
| Red blood cells ($x10^{12}$L)[§] | 3.1±0.6 | 2.7±0.6 | 2.4±0.2* |
| Platelets ($x10^9$L) | 455±135 | 464±125 | 454±102 |
| Hemoglobin (g/dL)[§] | 8.4±1.1 | 7.4±1.2* | 7.0±0.6* |
| Hematocrit (%)[§] | 26.5±3.9 | 23.4±3.6* | 20.8±2.9*** |
| Mean cell volume (fl) | 81.9±9.5 | 80.1±8.0 | 83.8±5.8 |
| MCH (pg) | 27.7±3.7 | 27.7±2.8 | 29.9±2.2 |
| Reticulocytes (%)[§] | 8.5±4.1 | 11.5±4.6 | 18.0±5.5***†† |
| Lactate dehydrogenase (IU)[§] | 476±148 | 608±125 | 655±309* |
| Total bilirubin (mg/dL)[§] | 2.5±1.3 | 4.2±2.9 | 5.9±2.6* |
| Blood viscosity ($mPa.s^{-1}$) | 6.9±2.3 | 6.4±1.9 | 8.1±2.6 |
| RBC deformability at 3 Pa (a.u)[§] | 0.19±0.06 | 0.15±0.05* | 0.09±0.03***† |
| RBC deformability at 30 Pa (a.u)[§] | 0.44±0.09 | 0.35±0.08** | 0.26±0.07*** |
| RBC aggregation index (%)[§] | 52.0±10.4 | 49.9±9.9 | 42.1±8.2 |
| RBC disaggregation threshold ($s^{-1}$)[§] | 237±74 | 280±78 | 347±176 |
| VOC rate[§] | 0.82±1.33 | 0.25±0.35 | 0.23±0.35 |
| ACS rate | 0.16±0.20 | 0.12±0.15 | 0.15±0.15 |

*Values represent mean ± SD. $SpO_2$: hemoglobin oxygen saturation; MCH: mean cell hemoglobin; VOC, vaso-occlusive crises; ACS: acute chest syndrome. Different from group with $SpO_2$ > 98% (*P<0.05; **P<0.01; ***P<0.001); different from group with 95 ≤ $SpO_2$ ≤ 98% (†P<0.05; ††P<0.01; †††P<0.001). [§]Variable with P<0.20 by ANOVA included in the multivariate analysis. [‡]Variable discarded from the multivariate analysis to avoid co-linearity.*

### Exercise-induced oxygen desaturation in SS and SC children

In SC children, there was no difference in any of the measured parameters when comparing the patients without exercise-induced oxygen desaturation to those with exercise-induced oxygen desaturation, except for the VOC rate, which was greater in the SC children exhibiting a reduction in exercise-induced SpO2 ($P<0.05$) (Table 2). There was no difference in RBC disaggregation threshold or estimated asthma frequency (*data not shown*) between the two subgroups ($P<0.20$ by Mann-Whitney test). Nevertheless, they were included in a binary multivariate

logistical model along with the VOC rate. The overall model was not statistically significant ($\chi^2$=4.084; df=3; *P*=0.253).

A similar comparison was made in the SS group and showed differences in gender distribution (*P*<0.05), total distance walked (*P*=0.05), percentage of predicted distance walked (*P*<0.05) and ACS rate (*P*<0.05) between children with and without exercise-induced oxygen desaturation (Table 3). The RBC disaggregation threshold showed no difference between the two subgroups, but *P* was less than 0.20 by Mann-Whitney test, justifying its inclusion in a binary multivariate logistical model that included gender as factor and percentage of predicted distance, RBC disaggregation threshold, and ACS rate as covariates. The distance walked was not included in the model to avoid co-linearity effects with the percentage of predicted distance walked. The overall model was highly significant ($\chi^2$=20.885; df=4; *P*<0.001). The percentage of predicted distance (OR: 1.13; 95%CI: 1.03-1.21; *P*<0.01), RBC disaggregation threshold (OR: 1.01; 95%CI: 1.002-1.019; *P*<0.05) and ACS rate (OR: 1.05; 95%CI: 1.008-1.101; *P*<0.05) were significantly associated with exercise-induced hemoglobin oxygen desaturation. The exclusion of SS children under HU therapy did not change the results obtained by univariate analyses (*data not shown*) except that the comparison of percentage of reticulocytes between the two groups gave *P*<0.20. A second binary multivariate logistical model was tested including the same parameter as in the first model plus percentage of reticulocytes. The overall model was still highly significant ($\chi^2$=21.92; df=4; *P*<0.001). The percentage of predicted distance (OR: 1.16; 95%CI: 1.034-1.309; *P*<0.05), RBC disaggregation threshold (OR: 1.01; 95%CI: 1.002-1.026; *P*<0.05) and ACS rate (OR: 1.09; 95%CI: 1.021-1.154; *P*<0.01) were significantly associated with EIHOD. Finally, a third binary multivariate logistical model was tested on the whole SS group (children with and without HU), and we forced the inclusion of HU therapy as a co-factor. The results obtained were comparable to those obtained with the first model (*data not shown*).

## Discussion

The present study shows that: 1) EIHOD is observed in both SC (18%) and SS (34%) children; 2) EIHOD is independently associated with the six-minute walk distance and RBC disaggregation threshold in SS children; 3) no predictor of EIHOD was found in SC children. In addition, we confirm that: 4) SC children have no hemoglobin oxy-

**Table 2. Comparison of 6MWT, hematologic, hemorheological, ACS rate and VOC rate parameters of SC children classified accordingly to their level of exercise-induced hemoglobin oxygen desaturation.**

| | $SpO_2$ reduction < 3 (n=40) | $SpO_2$ reduction ≥ 3 (n=9) |
|---|---|---|
| Sex ratio (M/F) | 23/17 | 5/4 |
| α-thalassemia (%) | 35 | 22 |
| Hydroxyurea (%) | 0 | 0 |
| Age (years) | 12.0±2.2 | 11.6±2.6 |
| Walked distance (m) | 489±88 | 515±97 |
| Percentage of predicted distance (%) | 73.2±12.5 | 77.8±11.6 |
| Fetal hemoglobin (%) | 3.0±3.1 | 2.4±1.9 |
| Leukocytes ($x10^9$L) | 7.3±3.0 | 7.4±2.1 |
| Red blood cells ($x10^{12}$L) | 4.5±0.6 | 4.4±0.5 |
| Platelets ($x10^9$L) | 280±135 | 291±140 |
| Hemoglobin (g/dL) | 11.1±1.1 | 11.4±0.6 |
| Hematocrit (%) | 33.2±3.0 | 32.9±1.8 |
| Mean cell volume (fL) | 71.3±6.1 | 73.3±4.5 |
| MCH (pg) | 25.2±2.5 | 26.2±1.7 |
| Reticulocytes (%) | 2.9±1.2 | 3.3±0.9 |
| Lactate dehydrogenase (IU) | 305±87 | 268±44 |
| Total bilirubin (mg/dL) | 1.8±0.6 | 1.4±0.04 |
| Blood viscosity ($mPa.s^{-1}$) | 8.6±2.1 | 8.0±1.5 |
| RBC deformability at 3 Pa (a.u) | 0.17±0.03 | 0.18±0.03 |
| RBC deformability at 30 Pa (a.u) | 0.45±0.05 | 0.45±0.06 |
| RBC aggregation index (%) | 44.4±8.7 | 46.6±7.2 |
| RBC disaggregation threshold ($s^{-1}$)[§] | 274±118 | 327±122 |
| VOC rate[§] | 0.23±0.41 | 0.37±0.29* |
| ACS rate | 0.02±0.03 | 0.08±0.12 |

*Values represent mean ± SD. $SpO_2$: oxygen saturation; MCH: mean cell hemoglobin; VOC: vaso-occlusive crises; ACS: acute chest syndrome. Different from group with $SpO_2$ reduction < 3% (*P<0.05; **P<0.01; ***P<0.001). [§]Variable with P<0.20 included in the multivariate analysis.*

**Table 3. Comparison of 6MWT, hematologic, hemorheological, ACS rate and VOC rate parameters of SS children classified accordingly to their level of exercise-induced hemoglobin oxygen desaturation.**

| | $SpO_2$ reduction < 3 (n=37) | $SpO_2$ reduction ≥ 3 (n=19) |
|---|---|---|
| Sex ratio (M/F)[§] | 15/22 | 13/6* |
| α-thalassemia (%) | 42 | 37 |
| Hydroxyurea (%) | 24 | 16 |
| Age (years) | 11.5±2.3 | 11.4±2.5 |
| Walked distance (m)[§] | 445±76 | 487±68 (*P*=0.05) |
| Percentage of predicted distance[§] | 67.7±11.6 | 74.4±9.8* |
| Fetal hemoglobin (%) | 9.2±6.9 | 7.3±5.8 |
| Leukocytes ($x10^9$L) | 11.3±2.9 | 10.8±2.8 |
| Red blood cells ($x10^{12}$L) | 2.8±0.6 | 2.9±0.6 |
| Platelets ($x10^9$L) | 460±128 | 453±124 |
| Hemoglobin (g/dL) | 7.8±1.3 | 7.9±1.0 |
| Hematocrit (%) | 24.4±4.3 | 25.1±4.1 |
| Mean cell volume (fl) | 82.5±8.6 | 79.6±8.3 |
| MCH (pg) | 28.3±3.1 | 27.5±3.5 |
| Reticulocytes (%) | 10.5±5.2 | 11.6±5.9≠ |
| Lactate dehydrogenase (IU) | 561±177 | 517±196 |
| Total bilirubin (mg/dL) | 3.5±2.5 | 3.7±2.5 |
| Blood viscosity ($mPa.s^{-1}$) | 7.1±2.2 | 6.5±2.2 |
| RBC deformability at 3 Pa (a.u) | 0.17±0.06 | 0.15±0.06 |
| RBC deformability at 30 Pa (a.u) | 0.39±0.11 | 0.36±0.11 |
| RBC aggregation index (%) | 49.8±11.1 | 50.0±9.2 |
| RBC disaggregation threshold ($s^{-1}$)[§] | 249±87 | 302.3±118 |
| VOC rate | 0.56±1.19 | 0.51±0.58 |
| ACS rate[§] | 0.11±0.16 | 0.21±0.19* |

*Values represent mean ± SD. $SpO_2$, oxygen saturation; MCH: mean cell hemoglobin; VOC: vaso-occlusive crises; ACS, acute chest syndrome. Different from group with $SpO_2$ reduction < 3% (*P<0.05; **P<0.01; ***P<0.001). [§]Variable with P<0.20 included in the multivariate analysis. [≠]Variable discarded from the multivariate analysis to avoid co-linearity.*

gen desaturation at rest whereas approximately 50% of SS children exhibit resting hemoglobin oxygen desaturation; 5) resting hemoglobin oxygen desaturation in SS children is independently associated with the level of hemolysis.

In this study, arterial blood gases did not confirm the reduction in arterial oxygen partial pressure and oxygen saturation. This could be considered to be a limitation as some studies have suggested that pulse oximetry overestimates arterial oxygen saturation in SCD.[42,43] Although pulse oximetry does not measure dysfunctional hemoglobin (i.e. methemoglobin or carboxyhemoglobin), and thus overestimates the true arterial oxygen saturation, it correlates well with co-oximetry (i.e. the gold standard method) in SCD.[3,4] The use of pulse oximetry to detect oxygen saturation has been proven to be very useful to predict the risks for stroke or elevated tricuspid regurgitation velocity in this population.[6,7,10]

The independent association found between the percentage of reticulocytes and resting hemoglobin oxygen desaturation in SS children is in accordance with several previous studies showing that the hemolytic rate is a predictor of resting oxygen desaturation in this population.[1,5,6,11,14,15] Kato *et al*. and Campbell *et al*. suggested that chronic hemolysis might be responsible for pulmonary vasculopathy which could cause ventilation-perfusion mismatching and hemoglobin oxygen desaturation at rest.[6,16] Several studies reported an independent association between the level of anemia and oxygen desaturation.[1,5,11,14] We also found that SS children with hemoglobin oxygen desaturation had a lower hemoglobin level than SS children without hemoglobin oxygen desaturation, but the different ordinal multivariate logistical models tested failed to demonstrate an independent association between the two parameters. The reasons for this lack of independent association are unknown, but Quinn et al. observed that the level of anemia explained only 5% of the arterial oxygen desaturation variability in SS/S$\beta^0$ thalassemia children, and suggested that anemia was not the main factor explaining the presence of hemoglobin oxygen desaturation at rest.[5] Nevertheless, further studies using primary markers of hemolysis, such as life span,[40] or the recent integrated hemolytic marker validated by Nouraie *et al*.,[44] are needed to definitively exclude a role of anemia in resting hemoglobin oxygen desaturation in SS patients.

The prevalence of EIHOD during the 6MWT was 2-fold higher in SS children compared to SC children, emphasizing the greater risk for exercise-related complications in the former population as transient hypoxemic/hypoxic episodes could impair RBC rheology and activate endothelial cells. Nevertheless, a small proportion of SC children (almost 20%) also experienced exercise-induced oxygen desaturation. Accurate screening by pulse oximetry during cardiopulmonary testing may be of benefit in SC patients to identify those who could be at greater risk for exercise-related complications. However, we found no association between the parameters investigated and EIHOD in the SC group, and the mechanisms of the reduction in hemoglobin saturation during exercise in this population remain unknown.

EIHOD was not related to resting lung dysfunction in SS children (*data not shown*). However, because we measured pulmonary function before and not after the 6MWT, we cannot exclude that exercise lung dysfunction plays a role in EIHOD in children with SCD. The independent association found between EIHOD and ACS rate in SS children contrasts with the finding from a previous larger study by Campbell and co-workers in children with various mixed SCD genotypes.[6] Further studies in larger cohorts are needed to specifically test this association.

The independent association between EIHOD and the highest percentage of predicted distance walked in SS children suggests that the magnitude of the physiological strain during the 6MWT plays a role in the occurrence of hemoglobin oxygen desaturation. This has also been reported in healthy athletes in whom the occurrence of transient EIHOD is dependent on the intensity at which they exercise.[45]

In a non-SCD context, blood rheological alterations have been suspected to participate to the occurrence of EIHOD.[27,28] The independent association between the elevated RBC disaggregation threshold and EIHOD in SS children reported in the present study also supports a role for blood rheology in EIHOD. A high RBC disaggregation threshold means that RBC aggregates are tightly bound.[20] This may increase flow resistance, promote arteriovenous shunts and disturb microcirculation at the entry of the pulmonary capillaries where RBC aggregates need to be fully dispersed before they can enter and negotiate small capillaries to promote adequate gas exchange between the lungs and RBC.[46]

A major limitation of the present study is the absence of a healthy control group. Campbell *et al*.[6] showed that 52% of sickle patients (SC, SS, Sβ thalassemia, SO$^{Arab}$ and other genotypes) and 24% of healthy controls (matched for age and ethnicity) had resting hemoglobin oxygen saturation of less than 99%, and 9% of patients *versus* no controls had resting hemoglobin oxygen saturation level of less than 95%. They also found that 8% of the sickle patients had a significant reduction in hemoglobin oxygen saturation below resting level (i.e. ≥3%) after a 6MWT while none of the controls had such a reduction.[6]

In conclusion, we confirm that hemoglobin oxygen desaturation at rest is common in SS children, but not in SC children, and is mainly associated with higher hemolysis. While EIHOD is more frequent in SS children, we show that it also occurs in a small percentage of SC children. Physiological strain during exercise and RBC aggregation properties are likely risk factors in the occurrence of exercise-induced hemoglobin oxygen desaturation in SS children.

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***Authorship and Disclosures***

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